PCR provided comparable results at the mRNA level. Treatment with 50-400 μM EGCG increased the expression of Nrf2 and HO-1 in HUVECs exposed to $PM_{2.5}$ in a dose-dependent manner. Western blotting reveals that pre-incubation with PD98059 (an ERK1/2 inhibitor) and SB203580 (a p38 MAPK inhibitor), but not SP600125 (a JNK inhibitor), abrogated EGCG-induced Nrf2 and HO-1 upregulation in HUVECs exposed to $PM_{2.5}$. In order to demonstrated that Nrf2 expression plays an important role in the EGCG-induced upregulation of Nrf2 and HO-1 and cell viability improvement Nrf2 shRNA transfected HUVECs and normal HUVECs were treated with EGCG for 30 minutes followed by 200μg/ml $PM_{2.5}$ for another 24 hours. Western blotting show that the efficiency of Nrf2 silencing was about 50% and the EGCG-induced upregulation of Nrf2 and HO-1 were abolished completely. Furthermore, Nrf2 silencing decreased the effect of EGCG on $PM_{2.5}$-induced HUVECs viability to 50%.

CONCLUSIONS In conclusion, the present study provides evidence that EGCG protects HUVECs from oxidative stress induced by high-dose PM2.5 through activation of the p38 MAPK and ERK1/2 pathways, which in turn increase the expression of Nrf2/HO-1.

GW26-e5346

Proteomics Analysis of Changes in Acetylated Proteins Expression Profiling in Right Appendages Tissue From Valvular Heart Disease Patients With Chronic Atrial Fibrillation

Tao Tu, Qiming Liu, Xuping Li, Zhengjiang Liu, Shenghua Zhou
Department of Cardiology, Sencond Xiangya Hospital, Central South University

OBJECTIVES The aim of the present study was to perform a proteomic analysis to compare the possible differences in the expression of acetylated proteins in right atrial appendages(RAA) from patients in sinus rhythm(SR) and in chronic AF. We will emphasis on the analysis of the regulatory role of acetylated proteins to the energy metabolic disorders in AF, and finally provide theory basis and potential therapy targets for intervention in AF.

METHODS The RAA samples were obtained as surgical biopsies at the time of the mitral valve surgery from valve disease patients with chronic AF (n=12) and SR (n=10). Specimens were pulverized under liquid-N2 into a fine powder, which was homogenized in a lysate buffer. We completed the acetyl-lysine enrichment of the obtain protein according to PTMScan acetyl-lysine enrichment protocol. The enriched acetyl-lysine peptides was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). We identified the function of all the acetylated proteins through searching the gene bank, and focused on analyzing main proteins associated with energy metabolism. We used the immune coprecipitation technology combined with Western blotting analysis to validate the expression of acetylated 2-oxoglutarate dehydrogenas (OGDH) and pyruvate dehydrogenase E1 component subunit beta (PDHB) in the RAA from SR and AF patients.

RESULTS We have detected 1570 different acetylated peptides in the RAA tissue from chronic AF patients, those peptides correspond to 140 unique proteins and 128 of them were effective acetylated protein related to cardiac tissue. 582 different acetylated peptides were detected in the RAA tissue from SR patients, those peptides correspond to 56 unique proteins and 48 of them were effective acetylated proteins related to cardiac tissue. 43 acetylated proteins were expressed in RAA of both SR and chronic AF patients. Among them, 22 were energy metabolism related acetylated proteins including many metabolic enzymes of Krebs cycle and oxidative phosphorylation process, 16 of them were up-regulated in RAA of AF patients. 14 cellular structure acetylated proteins were involved in cytoskeleton and myocardial contraction and 5 of them were up-regulated in RAA of AF patients. 2 acetylated histones were core component of nucleosome and histone H4 was upregulated in RAA of AF patients, and other 5 acetylated proteins were involved in cell growth, proliferation and gene expression, etc. The results of the validation trends are consistent with results identified by MS which demonstrated the reliability of the MS analysis in this study.

CONCLUSIONS The profile of acetylated proteins in AF patients was different from that in SR patients. The energy metabolism related acetylated proteins expressed in the RAA of chronic AF patients were mainly metabolic enzymes involved in multiple metabolic pathways, and the significant acetylation of those proteins participate in the regulation of impaired energy metabolism in right atrium during AF.

GW26-e5407

Postprandial Triglyceride-Rich Induced Adipogenesis Differentiation Is Dependent on Apolipoprotein E Carried on Lipoprotein

Mingyu Zhang, Yanhong Li, Ling Liu
the Second Xiangya Hospital, Central South University

OBJECTIVES Postprandial hypertriglyceridemia is closely related with obesity. Postprandially increased triglyceride-rich lipoproteins (TRLs) took an important role in adipocyte hyperplasia. The aim of this study was to investigate the effects of apolipoprotein E (ApoE) carried on postprandial TRLs on adipogenesis and the potential mechanisms underlying this.

METHODS Postprandial TRLs were isolated by density gradient ultracentrifugation from plasma in patients or mice at 4h after a high-fat meal. 3T3-L1 cells were cultured with various concentrations of humanTRLs (h-TRLs) (25, 50, 100, 150 μg/mL) in the presence or absence of 10 μg/mL insulin for two weeks, or with both 100 μg/mL h-TRLs and 10 μg/mL insulin for various days (0, 4, 7, 10, 14 days). To explore the effect of TRL-bound apoE in TRL-induced adipogenesis, 3T3-L1 cells were incubated with 100 μg/mL TRLs from wild type mice (WT-TRLs) or apoE knock-out mice (EKO-TRLs) and 10μg/mL insulin for 14 days. Oil-red O staining and expressions of adipogenesis markers were detected. Differentiating adipocytes were incubated with different kinds of TRLs labeled by Atto-565-NHS that emits red fluorescence. Then laser confocal microscopy was performed to determine the locations of TRLs to further investigate the effect of TRL-bound apoE on endocytosis of TRLs by differentiating adipocytes. Receptor associated protein (RAP), heparin or both were added to prevent the interaction between TRLs and LDLR family receptors, heparansulfate proteoglycan (HSPG) or both, respectively, to investigate the receptor-mediated pathway in the endocytosis of TRLs. Real-time PCR and western blot were used to detect the expressions of endocytic receptors associated with apoE during TRLs-induced adipogenesis.

RESULTS H-TRLs with insulin (10 μg/mL) successfully induced 3T3-L1 to form mature adipocytes. Both protein and mRNA expressions of adipocyte fatty acid binding protein 2 (aP2) and peroxisome proliferator activated receptor γ (PPAR-γ) increased not only along with the increase of TRLs concentration ($P<0.05$), but also with the treating time of 100μg/mL TRLs ($P<0.05$). With the assistance of insulin, WT-TRLs induced 3T3-L1 to produce lipid droplets, whereas EKO-TRLs did not. Confocal microscopy analysis clearly revealed that red fluorescence could be seen within the differentiating adipocytes treated with h-TRLs or WT-TRLs, but not in those with EKO-TRLs. Compared with control group, RAP markedly reduced red fluorescence within the differentiating adipocytes, while heparin had little impact. The protein level of LRP1 showed upward trend with the increase of TRLs concentrations. Compared with undifferentiated preadipocytes, the strong expression of LRP1 protein was detected throughout 14 days.

CONCLUSIONS Postprandial TRLs with insulin induced adipogenesis differentiation in dose- and time-dependent manner. Lipoprotein-bound apoE was required in TRLs-induced adipogenesis and the endocytosis of TRLs by the differentiating adipocytes that internalized TRLs via LDLR family members, probably LRP1.

GW26-e0383

Effects of PKC Activity on the Adhesion Reaction of Atherosclerosis

Suining Xu, Jie Zhu, Yingpeng Ding, Jinchuan Yan, Zhongqun Wang
Department of Cardiology, Affiliated Hospital of Jiangsu University

OBJECTIVES To investigate the effects of protein kinase C(PKC) activity on the adhesion reaction of atherosclerosis.

METHODS The present study consisted of an in vivo investigation and four in vitro investigations. In the in vivo investigation, 24 New Zealand rabbits were induced into atherosclerosis by the administration of high cholesterol diet for 12 weeks. The changes of PKC activity in atherogenesis were quantitated by PepTag®Assay for Non-Radioactive Detection and the distribution of PKCα in plaques was detected by immunohistochemistry staining; In in vitro experiments, PKC activity in modified low density lipoprotein (LDL)- loaded cells (including HASMC smooth muscle cells, THP-1 monocytes/macrophages and HAEC endothelial cells which play a key role in atherogenesis) were detected. Furthermore, the mechanisms of PKC, ICAM-1, I-κBα and ezrin in the adhesion reaction of endothelial cells with monocytes were explored.

RESULTS PKC activity rose significantly in the atherosclerotic aorta of New Zealand Rabbits which were fed by high cholesterol diet for